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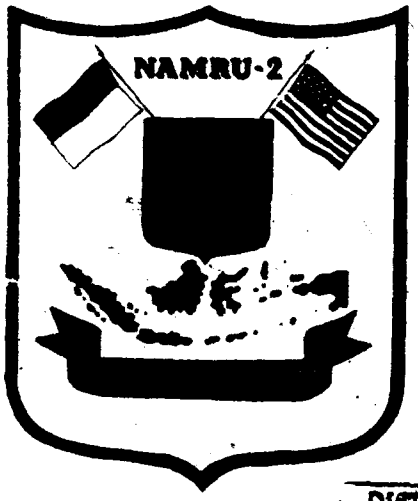
REPORT NO. TR-1064



UNITED STATES NAVAL MEDICAL RESEARCH UNIT NO. TWO
APO SAN FRANCISCO, CALIFORNIA 96528 – 5000

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
BETHESDA, MARYLAND

ADMINISTRATIVE INFORMATION

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This work was supported by funds provided by the Naval Medical Research Unit No. 2 for Work Unit 041.09-002-5037 and by the Indonesian Ministry of Health.

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ISOLATION OF *SALMONELLA TYPHI* FROM STANDARD WHOLE-BLOOD CULTURE *VS* BLOOD-CLOT CULTURES

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INTRODUCTION

The clot culture technique using streptokinase added to sodium taurocholate nutrient broth medium (bile broth) was reported by Watson (1978) to be superior to the whole blood culture system for the isolation of *S. typhi*. The culture of blood clots increased the number of positive isolations and decreased the time of incubation required to obtain a positive culture. Watson (1954) stated that this was presumably due to the removal of serum which contains bactericidal factors, especially antibody and complement.

This study was supported through funds provided by the Naval Medical Research and Development Command, Navy Department Work Unit MR-041.09-002-5037.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Navy Department or the U.S. Naval Service at large or the Indonesian Navy Medical Service.

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Watson (1978) also reported that streptokinase provides rapid lysis of the blood clot, thus releasing the organisms trapped within the clot and freeing them from the bactericidal activity of serum factors adsorbed to the fibrin network.

Escamilla *et al.*, (1984) stated that whole blood culture systems are not inferior to the clot cultures. They found in cultures from 68 patients positive for *S. typhi*, the isolation rate of a whole blood culture system was 87% compared to 68% for clot cultures. Escamilla (1984) used 10% oxgall for culturing blood clots, whereas Watson (1954, 1978) used sodium taurocholate nutrient broth. In several laboratories (Escamilla *et al.*, 1984; Mikhail *et al.*, 1983; Rockhill *et al.*, 1980), 10% oxgall is used quite commonly for isolating *S. typhi* and *S. paratyphi* from venous blood of patients with enteric fever. In this study, we evaluated the use of bile broth and 10% oxgall in the clot culture technique and compared them to whole blood culture systems using 3 ml and 8 ml blood in a 1:4

and 1:10 blood to medium ratio, respectively.

MATERIALS AND METHODS

Blood culture media: Bile broth was prepared by adding 5 grams of sodium taurocholate (BBL, Cockeysville, MD) to 1 liter of sterile nutrient broth, pH 7.6 (Difco Laboratories, Detroit, MI). Fifteen ml was dispensed into 30 ml serum bottles and steamed for 1 hour. Oxgall medium was prepared by dissolving oxgall (Difco) to a final concentration of 10% in distilled water. The oxgall medium was then heated to boiling and dispensed in 9 ml and 15 ml quantities in 30 ml serum bottles. Additionally, 72 ml amounts of oxgall medium were dispensed in 100 ml serum bottles. After dispensing, all oxgall medium bottles were steamed for 1 hour.

Specimens and culture systems: Venous blood was drawn from patients hospitalized with a tentative diagnosis of typhoid fever admitted to Rumah Sakit Persahabatan (Friendship Hospital) from March 1984 through March 1986. The study was conducted during two different periods. During the first period (March 84 – August 85) clots were cultured in 10% oxgall solution (CLOX) and during the second period (August 85 – March 86) in bile broth (CLBB). Whole blood was cultured in 10% oxgall throughout the study.

Three ml and 8 ml of whole blood was inoculated at the bedside into 9 ml (1:4 dilution = blood culture (BC) 1:4) and 72 ml (1:10 dilution = BC 1:10) of 10% oxgall, respectively. An additional 8 ml of blood was allowed to clot in a sterile tube at room temperature (about one hour) and was then centrifuged at 1000 x g for 15 minutes. The serum was removed and the clot was aseptically placed in 15 ml sterile medium containing 1500 units of freshly prepared streptokinase (Kabikinase, Pharmacia Laboratories, Piscataway, NJ). At the hospital, Amies transport medium was inoculated with rectal swabs from each patient. After receipt in the laboratory, the rectal swabs were used to inoculate MacConkey agar (Difco) and Salmonella–Shigella (SS) (Difco) agar. The swabs were then placed in mannitol selenite broth (MSB) (Difco) for enrichment. After over-night incubation at 37°C, swabs from MSB were used to inoculate SS agar which was then incubated at 37°C for an additional 18–20 hours. Samples from the venous blood and blood clot cultures were used to inoculate desoxycholate citrate lactose sucrose (DCLS) agar (Difco), daily for one week, and again after 14 days and 21 days of incubation. Bacterial growth was identified according to the standard culture methods (Edwards and Ewing, 1972).

RESULTS

During the first period of the study, blood, clots and rectal swabs were collected from 241 patients. Of these patients, 97 were positive for *Salmonella typhi*. During the second period, specimens were obtained from 222 patients. *S. typhi* was isolated from 87 of these patients. Table 1 shows the recovery of *S. typhi* from BC 1:4, BC 1:10, CLOX, CLBB and from rectal swabs, during the first and second period of the study. As shown in Table 1, *S. typhi* isolation rate from clots in 10% oxgall medium (CLOX) was 57% whereas the isolation rate from clots in bile broth (CLBB) was 58%. Table 2 shows the cumulative positive cultures in relation to the day of incubation when the culture became positive. The blood-culture systems required

Table 1

Summary of *S. typhi* isolation in whole blood culture systems, clot culture in 10% oxgall (CLOX), clot culture in bile broth (CLBB) and rectal swab cultures.

Total isolates	Number of *S. typhi* isolated (percentage of total)				
	BC 1:10	BC 1:4	CLOX	CLBB	RS
97	66 (68)[a*]	54 (56)[b]	55 (57)[b]	ND	47 (49)
87	65 (75)[a]	54 (62)[b]	ND	50 (58)[b]	41 (47)

* Percentages followed by different letters are significantly different by X^2 test ($p \leq 0.02$).

7 days to yield all positive isolates using these methods, whereas the clot culture system using CLOX required 14 days to identify all positives. Also, on day 1, 64% of BC 1:10 and 56% of BC 1:4 were positive, but only 36% of the CLOX clot cultures and 46% of the CLBB clot cultures were positive.

The total blood clot culture positives in bile broth (CLBB) at day 4 was slightly greater than the positives in oxgall (CLOX) on the same day. These were 92% and 89%, respectively.

Of 184 *Salmonella typhi* isolated (Table 2), BC 1:10 with 71% (131/184) positive cultures showed the highest recovery rate, whereas BC 1:4 with 59% (108/184), showed nearly the same rate as the CLOX clot cultures with 57% (55/97) and CLBB clot cultures with 58% (50/87).

DISCUSSION

The results indicate that regardless of the medium being used (10% oxgall or bile broth, both supplemented with streptokinase) the clot culture system isolation rate for *S. typhi* does not exceed that of whole blood culture system (BC 1:10 or BC 1:4). There was no advantage of bile broth which showed an isolation rate of 58% for *S. typhi*, when compared to 10% oxgall which showed a rate of 57%. However, our data (Table 2) shows that during the first four days of incubation CLBB was slightly faster in showing positive *S. typhi* growth than was CLOX. The mean time for detection of a positive culture from CLBB was 2.80 days as compared to 2.56 days for CLOX. This could be caused by the nutritional content of the two different systems. Oxgall does not have the nutrient components that will stimulate growth whereas bile broth medium contains nutrient broth that will promote organism growth once released from the clot trap.

Watson (1978) showed that blood clot cultures in bile broth were 28% more sensitive than whole blood culture systems (8 ml blood in 50 ml bile broth). In contrast to his reports, we found the BC 1:10 result was significantly better than those of the clot cultures or the BC 1:4. Our results differ

Table 2

Isolation of *S. typhi* from whole blood culture (BC 1:4 and BC 1:10) and clot culture (CLOX) in 10% oxgall and clot culture (CLBB) in bile broth shown by day of incubation when the culture became positive.

Day of incubation	BC 1:4 (n = 108)	BC 1:10 (n = 131)	CLOX (n = 55)	CLBB (n = 50)
	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)
1	64 (59)	79 (60)	20 (36)	23 (46)
2	21 (79)	12 (78)	15 (64)	15 (64)
3	17 (94)	16 (90)	8 (78)	9 (90)
4	4 (98)	7 (95)	6 (89)	1 (92)
5	1 (99)	2 (97)	3 (95)	0 (92)
6	0 (99)	4 (100)	0 (95)	2 (96)
7	1 (100)	–	2 (98)	0 (96)
14	–	–	1 (100)	2 (100)

markedly from those of Watson (1978), however our findings are consistent with the observations reported by Escamilla *et al.*, (1984) and by Simanjuntak (in press). Although we have extended the time of incubation for both blood and blood clot culture systems to 21 days, we failed to get any additional positive results from specimens after day 14. Three of 105 positive cultures were not found positive until day 14. The blood culture systems showed no additional positives after day 7.

The clot culture is valuable when specimens are required for serologic testing such as the Widal agglutination test. One tube may be drawn and the serum used for the serological studies while the remaining clot may be used for a blood culture. It must be remembered that when isolating *S. typhi* is of prime importance, the whole blood cultures (BC 1:10) is preferable and recommended above either clot culture technique, or BC 1:4. Also, there is no significant difference between the use of CLBB or CLOX for clot cultures.

SUMMARY

The use of 10% oxgall and bile broth medium, both supplemented with freshly prepared 100 u/ml streptokinase, for isolating *Salmonella typhi* by clot culture technique was evaluated and compared against whole blood culture systems (3 ml blood in 9 ml media and 8 ml blood in 72 ml media). These gave a 1:4 and 1:10 blood to medium ratio, respectively. Clot cultures in 10% oxgall (CLOX) gave a 57% positive isolation rate for *S. typhi*. A similar result was obtained from clot cultures in bile broth medium

(CLBB). A total of 184 samples identified as positive for *S. typhi* were tested. There was no significant difference between the use of 10% oxgall or bile broth medium when used for clot culture. The whole blood culture systems still showed a significantly better rate of isolation than the clot culture methods.

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NAMRU-2-TR-1064	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION U.S. Naval Medical Research Unit No. 2 6b. OFFICE SYMBOL (If applicable) NAMRU-2	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) APO San Francisco, California 96528-5000	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION Naval Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable) NMRDC	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 63706N; PROJECT NO: M0095PN; TASK NO: M0095PN002; WORK UNIT ACCESSION NO: 5037

11. TITLE (Include Security Classification)
(U) Isolation of Salmonella typhi from standard wholeblood culture vs blood-clot cultures.

12. PERSONAL AUTHOR(S) Periska Tjaniadi, Edward M. Lane, Murad Lesmana, David C. Edman and Deskian Kostermans

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Technical Report	FROM 1984 TO 1986	1988 December	5

16. SUPPLEMENTARY NOTATION
Published in the Journal of Southeast Asian J. Trop. Med. Public Health, 19(4):623-627

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Salmonella typhi; Whole blood culture; Blood-clot culture; Isolation of S. typhi; Reports

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

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Keywords: cultures, kidney; clot cultures;

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL RAM I: Groyes, M.	22b. TELEPHONE (Include Area Code) 301/663-7567 22c. OFFICE SYMBOL NMRDC

DD Form 1473, JUN 86 *Previous editions are obsolete.*
S/N 0102-LF-014-6603

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UNCLASSIFIED